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Use of string test and stool specimens to diagnose pulmonary tuberculosis☆

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SUMMARY

Background—The Xpert MTB/RIF (MTB/RIF) test has advanced the field of tuberculosis (TB) diagnostics; however, depending on age and HIV status, 10–85% of individuals with presumed pulmonary TB (PTB) are unable to produce sputum.

Methods—The feasibility of using MTB/RIF and culture on stool and string test specimens from 13 adult patients with presumed PTB was studied.

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Conflict of interest: EJB is Professor Emerita at Stanford and also Technical Director at Cepheid, and provided assistance with stool processing protocols and study design, but not with data collection or data analysis. The study concept and goals were conceived with no input from Cepheid. Cepheid donated the cartridges for the project, but we did not receive any funding or other incentives. No other authors have conflicting interests.

Results—The string test was well tolerated with a median Wong Baker Faces score of 2. The string test had 100% sensitivity and specificity by MTB/RIF and 87.5% sensitivity and 100% specificity by culture. In stool, *Mycobacterium tuberculosis* DNA was detected in all cases of culture-confirmed PTB.

Conclusion—The string test and stool provide diagnostic specimens that warrant further investigation.

Keywords

Tuberculosis; String test; Stool; Xpert MTB/RIF

1. Introduction

Until recently, the diagnosis of pulmonary tuberculosis (PTB) has relied on sputum smear microscopy and culture. Unfortunately traditional culture requires at least 10–14 days and microscopy is highly variable with 50–80% sensitivity in PTB patients without HIV infection and 40–50% in those with HIV infection.[1,2] The Xpert MTB/RIF (MTB/RIF) test is an automated nucleic acid amplification technology (NAAT) that detects *Mycobacterium tuberculosis* DNA and rifampicin resistance in 2 h with an overall diagnostic sensitivity of 88%.[3]

While MTB/RIF has improved the rapidity of TB diagnostics, limitations persist. While more sensitive than smear, MTB/RIF is less sensitive than culture, likely related to its lower limit of detection of 131 CFU/ml compared to 10–100 CFU/ml for sputum culture.[4] MTB/RIF fails to detect one-third of adult and 45% of pediatric smear-negative, culture-positive cases.[3,5] Further, depending on age and HIV status, 10–85% of patients with presumed TB are unable to produce sputum specimens.[6–8] Most of these individuals undergo further invasive diagnostic studies such as bronchoscopy, which is often not available in resource-limited settings. There is a need for alternative specimen collection methods that can increase microbiologically confirmed cases.[3]

The benefit of using gastrointestinal specimens for the diagnosis of PTB has been known since 1898, when Meunier performed the first gastric lavages in children. While the procedure was mainly reserved for pediatric patients unable to expectorate,[9] in 1937 Gullbring and Levin reviewed a large series of adults in which gastric lavage detected 94% of cavitary TB and 55% of sputum-negative PTB.[10] Of interest, in “closed, benign apical fibrosis”, currently designated as latent TB infection with cavitary radiography, 32% of gastric lavages found TB bacilli.[10]

Frustrated by a progressive and extensive case of PTB with repeated negative sputum microscopy, in 1933 Ulmar and Ornstein performed two gastric lavages, finding TB in both. Inspired by this success, and building upon previous work by Hudson using iodized oil to slow the peristaltic activity of the bronchial tree,[11] Ulmar and Ornstein demonstrated the transition of bronchial fluid into the stomach by injecting iodized poppy-seed oil into the tracheobronchial tree and performed serial roentgenograms.[12] Recently, with the advent of

molecular diagnostics not requiring viable organisms, there has been a resurgent interest in novel gastrointestinal sources.[13,14]

Vargas et al. studied the utility of the string test in HIV-infected individuals with presumed PTB. The string test was superior to induced sputum in 160 cases, diagnosing 14 culture-confirmed cases compared to eight for induced sputum ($p = 0.03$).[8] Coupled with advances in molecular diagnostics, we sought to evaluate the feasibility of gastrointestinal samples (string test and stool[14]) using MTB/RIF.

2. Case report

In March 2014, a 64-year-old male presented with 2 weeks of diffuse, intermittent chest pain associated with mild dyspnea, but without cough. He had lost 10 kg in weight over the past year. He had a past medical history of hypertension and diabetes mellitus, and a history of colon cancer status post resection and chemotherapy. Colonoscopy and computed tomography (CT) examination of the chest, abdomen, and pelvis performed in Bangladesh were negative for residual cancer. He had emigrated from Bangladesh 1 year previously, was a former smoker with a 45-pack-year history, and denied alcohol or illicit drug use. A physical examination was unremarkable aside from cachexia. His hemoglobin was 10.8 g/ml and creatinine clearance was 51 ml/min; other laboratory studies were normal. He reported a negative tuberculin skin test pre-immigration. His chest radiograph showed scattered bi-apical hazy opacities and nodules. Chest CT showed mild emphysematous changes and multiple bi-apical ill-defined nodules, the largest nodule measuring 2.2 cm. He had three acid-fast bacillus (AFB) smear-negative induced sputum tests. Bronchoscopic alveolar lavage (BAL) was AFB smear-negative. As part of a feasibility diagnostic study, *M. tuberculosis* DNA was detected in both string test and stool. The patient was started on anti-TB therapy (ATT). The BAL fluid and string test became culture-positive 3 weeks later.

3. Methods

From August 2013 to March 2014, 13 participants were enrolled at Ben Taub General Hospital (Houston, Texas, USA) with presumed PTB; these patients had received <72 h of ATT. As per routine clinical care, liquid culture (BACTEC MGIT 960) and phenotypic drug susceptibility testing was performed on *M. tuberculosis* isolated from the clinical samples acquired from expectorated or induced sputum. The string test was performed in the morning, after an overnight fast.[8] The string capsule was swallowed, with the trailing end taped to the cheek. Four hours later it was removed by gentle traction, placed in 2 ml of phosphate buffered saline, vortexed, and 1 ml was used for MTB/RIF testing and culture.

Stool specimens were processed by two methods that do not require centrifugation. In the sugar floatation method, 0.5 g of stool was mechanically emulsified in 10 ml 50% Sheather's solution. Then the solution was manually inverted, filtered through funnel paper, and allowed to settle by gravity for 1 h. The top supernatant (0.5 ml) was incubated in a 1:1 ratio with MTB/RIF manufacturer-supplied NaOH and isopropanol decontamination Sample Reagent for 30 min and then analyzed by MTB/RIF.[15] In the magnetic bead method, 0.5 g of stool was first mixed with 1 ml of the NaOH/isopropanol Sample Reagent solution,

filtered using coarse material, mixed with the magnetic MicroSense Beads (Microsens Medtech Ltd, London, UK) and washed twice in accordance with the manufacturer's instructions before being tested by MTB/RIF.

This study was performed in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the institutional review boards (IRB) of Baylor College of Medicine and Harris Health System for Ben Taub General Hospital. All participants provided IRB approved written informed consent for the collection of samples and subsequent analysis.

4. Results

Of the 13 participants with presumed PTB, eight had culture-confirmed PTB. Two subjects had non-tuberculous mycobacteria pulmonary disease and three had other etiologies for their symptoms. Two of the eight patients had induced or expecto-rated sputum smear-negative, culture-positive disease. All 13 participants had successful string tests and 10 were able to provide stool samples (see Table 1). Four participants (30%) underwent bronchoscopic lavage as requested by their clinical teams.

The string test placement and 4-h evaluation was well tolerated. Minor discomfort occurred during string removal. The median Wong–Baker Faces pain score was 2, with a range of 0 to 6 (score range of zero meaning no pain to 10 being the worst pain). MTB/RIF on string fluid had 100% sensitivity and specificity, whereas culture of string fluid had 87.5% sensitivity and 100% specificity. The string culture and MTB/RIF assay were positive in both cases of smear-negative disease.

Seven of eight participants with PTB were able to provide stool within 72 h of ATT initiation. *M. tuberculosis* DNA from stool was detected in seven of seven PTB subjects, using either the sugar flotation or magnetic bead procedure. The sugar flotation PCR detected four of the seven (57%) participants, while magnetic bead concentration PCR detected six of the seven (85%) participants. Stool culture was positive in four of seven samples. Five of 10 (50%) stools processed with sugar flotation yielded invalid results compared to one of 10 (10%) by magnetic beads.

Rifampicin resistance was detected by sputum, string, and stool in one case. MTB/RIF and phenotypic drug susceptibility had 100% concordance with all specimen types.

5. Discussion

Given the limitations of current methods, the feasibility and tolerability of the string test and stool for diagnosing PTB were evaluated. The string test was found to be well tolerated and provided a satisfactory sample for diagnosing PTB when used with Xpert MTB/RIF and culture. The string test analysis with MTB/RIF had 100% concordance with sputum culture, including detecting both cases of smear-negative, culture-positive disease and the one case of rifampicin resistance. A previous study using liquid and solid media in 160 patients with HIV infection reported the string test to be superior to sputum.[8]

The findings in this feasibility report suggest that the diagnostic yield and utility of string test and stool should be compared to sputum, the current standard of care, in a larger diagnostic study. The string test is well-tolerated, low-risk, and simple to perform. In patients who are unable to produce sputum, these methods broaden the samples available to establish a TB diagnosis and potentially avoid more invasive procedures.

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Table 1

Comparison of sputum, string test, and stool by smear, culture, and MTB/RIF

Sputum smear status	Sputum culture	Sputum Xpert	String Xpert	String culture	MicroSense Bead Xpert	Sugar Xpert	Stool culture	Wong–Baker Faces
Mycobacterium tuberculosis culture-positive, smear-positive								
2–4+	*M. tuberculosis*	Positive	Positive	Positive	Positive	Positive	Negative	1
4+	*M. tuberculosis*	Positive	Positive	Positive	Positive	Invalid	Positive	6
1+	*M. tuberculosis*	Positive	Positive	Negative	Negative	Positive	Negative	2
3+	*M. tuberculosis*	Positive	Positive	Positive	Positive	Invalid	Positive	2
3+	*M. tuberculosis*	Positive	Positive	Positive	Positive	Positive	Positive	2
1+	*M. tuberculosis*	Positive	Positive	Positive	Positive	Positive	Positive	2
Mycobacterium tuberculosis culture-positive, smear-negative								
Negative	*M. tuberculosis*	Positive	Positive	Positive	UTP			4
Negative	*M. tuberculosis* [a]	Positive	Positive	Positive	Positive	Invalid	Negative	0
Mycobacterium tuberculosis culture-negative								
1+	*M. avium*	Negative	Negative	Negative	Negative	Invalid	Positive	1
3+	*M. kansasii*	Negative	Negative	Positive	Negative	Negative	Positive	2
Negative	Negative[b]	UTP	Negative	Negative	UTP			2
Negative	Negative[c]	Negative	Negative	Negative	UTP			0
Negative	Negative[d]	Negative	Negative	Negative	Invalid	Invalid	Negative	2

UTP, unable to produce.

[a] Smear and culture were negative from induced sputum. Bronchoscopic lavage fluid was smear-negative, but culture-positive.

[b] Final diagnosis of *Streptococcus milleri* aspiration related to alcohol intoxication.

[c] Final diagnosis unknown/undetermined and symptoms resolved.

[d] Final diagnosis of interstitial lung disease related to dermatomyositis.